

LEGAL NOTICE

This report was prepared by Ontario Hydro and, while effort has been made to assure the accuracy and applicability of the material contained herein, neither Ontario Hydro nor any person acting on its behalf:

- (a) makes any warranty, express or implied with respect to the use of any information, apparatus, method or process disclosed in this report or that such use may not infringe privately owned rights; or
- (b) assumes any liabilities with respect to the use of, or for damages resulting from the use of, any information, apparatus, method or process disclosed in this report.



ontario hydro research division

COMPLIMENTARY COPY

Please Credit Ontario Hydro Research if
Reproduced in Whole or in Part

Translocation of the Growth Regulator Uniconazole (XE-1019) in Silver Maple Saplings After Trunk Injection in the Spring

G.P. Arron
Associate Research Scientist
Biological Research Section
Chemical Research Department

ABSTRACT

Silver maple saplings were trunk injected with the growth regulator uniconazole (XE-1019) (^{14}C -labelled) in May 1987. After 500 days most ^{14}C activity remained around the injection site. Approximately 7.1 and 2.3% of the total ^{14}C activity applied was found in senescing foliage harvested in the fall of 1987 and 1988, respectively. About half this ^{14}C activity was associated with metabolites and not uniconazole.

job	file	date	report no.
34-528-090	834.1	May 18, 1989	89-60-K



ontario hydro research division

EXECUTIVE SUMMARY

G.P. Arron
Biological Research Section
Chemical Research Department

Growth regulators, such as paclobutrazol, flurprimidol and uniconazole, have been shown to inhibit tree growth in a number of studies in the U.S. and Canada. Application of such products to the trees under distribution lines which are trimmed by Ontario Hydro on a regular basis might reduce their rate of regrowth and decrease the frequency of trimming. Of the various application methods available (eg trunk injection, bark banding, basal drench, soil injection, foliar spray) trunk injection offers advantages over other methods in that the amount applied is precise, chemical contact with non-target plants is prevented and environmental residues are reduced.

Following trunk injection of growth regulators there is no direct release of the products into the environment. The growth regulators are translocated to the foliage and young shoots and are thought to remain there until the fall. After leaf drop the growth regulator (and/or metabolites formed from them) enter the soil system surrounding the woody plant. Microbial activity in the leaf litter/soil will result in further breakdown of the growth regulator and/or metabolites. Such a mechanism of release of growth regulator into the immediate plant environment is thought to be the primary path. The proportion of growth regulator, applied by trunk injection, that follow this path each year has not been estimated.

The translocation studies performed to date with the growth regulators paclobutrazol, flurprimidol and uniconazole have used young plant material and were short-term, terminating forty-two days or less after initiation. The present study was conducted in the field for 500 days. Trunk injection of silver maple saplings with ^{14}C -uniconazole in May 1987 resulted in an increase in chlorophyll pigmentation in the leaves and the appearance of stacked leaves in the 1988 growing season. However, such responses were non-uniform within saplings. No large-scale inhibition of sprout growth was apparent, although sprout growth was inhibited in 1988, but only when median sprout lengths were considered and not mean sprout lengths. Over the 1987 growing season there was an increase in the ^{14}C activity detected in the foliage. There was a similar increase in 1988, but the maximum concentration observed was only about one third that reached in 1987. Approximately 7.1 and 2.3% of the total ^{14}C activity injected into the saplings was present in senescing foliage harvested in the fall of 1987 and 1988, respectively. As the foliage

job	file	date	report no.
34-528-090	834.1	May 18, 1989	89-60-K

turned from green/yellow to yellow in the fall of 1988 there was no apparent withdrawal of ^{14}C activity from the leaves. In foliage collected in the fall of 1987 and 1988 about half of the ^{14}C activity detected was associated with metabolites and not uniconazole. The lack of an increase in the ^{14}C activity in metabolites suggests that metabolism of uniconazole occurred in the foliage rather than in the stem around the injection site. At the time of injection, and 134 and 500 days later, most of the ^{14}C activity detected in the saplings was around the injection site, with little in the stem between the injection site and the foliage.

GPA.
DRE

TABLE OF CONTENTS

	<u>Page</u>
1.0 INTRODUCTION	1
2.0 MATERIALS AND METHODS	2
2.1 Plant Material	2
2.2 Injection Procedure	3
2.3 Sample Collection	3
2.4 Determination of ^{14}C content of foliage, stem and root samples	3
2.5 Calculation of uniconazole concentration in foliage and sprout samples	5
2.6 Separation of ^{14}C -uniconazole and metabolites	5
2.7 Determination of leaf colour	6
3.0 RESULTS AND DISCUSSION	6
3.1 Effect of trunk injection of ^{14}C -uniconazole on the growth of silver maple saplings in the 1987 and 1988 growing seasons	6
3.2 ^{14}C activity in the foliage of silver maple saplings in 1987 and 1988 following trunk injection with ^{14}C -uniconazole (foliage uptake experiment)	11
3.3 ^{14}C activity in the stem, roots and foliage of silver maple saplings in 1987 and 1988 following trunk injection with ^{14}C - uniconazole (harvest experiment)	18
3.4 Metabolism of ^{14}C -uniconazole in foliage collected from silver maple saplings in the fall of 1987 and 1988	27
4.0 CONCLUSIONS	30
ACKNOWLEDGEMENTS	31
LITERATURE CITED	32
APPENDICES	34
DISTRIBUTION	last page

LIST OF TABLES

	<u>Page</u>
Table 1 Effect of trunk injection of ^{14}C -uniconazole on the number of sprouts formed on silver maple saplings in the 1987 and 1988 growing seasons	9
Table 2 Effect of trunk injection of ^{14}C -uniconazole on the length of sprouts formed on silver maple saplings in the 1987 and 1988 growing seasons - mean values	10
Table 3 Effect of trunk injection of ^{14}C -uniconazole on the length of sprouts formed above the injection site on silver maple saplings in the 1987 and 1988 growing seasons - median values	12
Table 4 Proportion of ^{14}C activity found in the foliage of silver maple saplings trunk injected with ^{14}C -uniconazole (foliage uptake experiment)	14
Table 5 Proportion of ^{14}C activity found in the foliage of silver maple saplings trunk injected with ^{14}C -uniconazole (harvest experiment)	17
Table 6 Change of colour and ^{14}C activity in foliage collected 498 and 512 days after injection of silver maple saplings with ^{14}C -uniconazole	19
Table 7 Stained wood found in silver maple saplings harvested 134 and 500 days after injection with ^{14}C -uniconazole or isopropyl alcohol (control)	21
Table 8 Retrieval of ^{14}C activity from harvested whole silver maple saplings following trunk injection with ^{14}C -uniconazole and proportion of ^{14}C activity in foliage and selected stem samples	24
Table 9 Concentration of uniconazole, calculated from ^{14}C activity data, in foliage and sprout growth samples collected from silver maple saplings 0, 17, 134 and 500 days after trunk injection with ^{14}C -uniconazole (harvest experiment)	26
Table 10 Distribution of ^{14}C activity in extracts of foliage from silver maple saplings trunk injected with ^{14}C -uniconazole (foliage uptake experiment)	28
Table 11 Distribution of ^{14}C activity in extracts of foliage from silver maple saplings trunk injected with ^{14}C -uniconazole (harvest experiment)	29

LIST OF FIGURES

	<u>Page</u>
Figure 1 Foliage, stem and root tissue samples collected on May 25th, 1987 from silver maple saplings immediately after injection with ^{14}C -uniconazole	4
Figure 2 Appearance of silver maple saplings in the 1988 growing season	7
Figure 3 Appearance of foliage collected from silver maple saplings in the 1988 growing season	7
Figure 4 Increase in ^{14}C activity in foliage collected in 1987 and 1988 from silver maple saplings trunk injected with ^{14}C -uniconazole on May 25, 1987	13
Figure 5 Distribution of ^{14}C activity in stem, root and foliage samples from silver maple saplings following trunk injection with ^{14}C -uniconazole on May 25, 1987	22
Figure 6 Distribution of ^{14}C activity (expressed as Bq/g dry weight) in stem, root and foliage samples harvested 500 days after trunk injection of silver maple saplings with ^{14}C -uniconazole	25

LIST OF APPENDICES

Appendix A	Dry weight of 30 leaf samples collected in 1987 and 1988 from silver maple saplings trunk injected with ^{14}C -uniconazole	34
Appendix B	Proportion of ^{14}C activity found in the foliage of silver maple saplings trunk injected with ^{14}C -uniconazole (foliage uptake experiment) - sapling values	35
Appendix C	Proportion of ^{14}C activity found in the foliage of silver maple saplings trunk injected with ^{14}C -uniconazole (harvest experiment) - sapling values	36



ontario hydro research division

To Dr. D. Mills
Director of Research

Translocation of the Growth Regulator Uniconazole (XE-1019) in Silver Maple Saplings After Trunk Injection in the Spring

1.0 INTRODUCTION

Approximately 1 million trees under or beside distribution lines are trimmed or removed by Ontario Hydro every year. The frequency with which trees are trimmed depends on factors such as species, location, orientation to line and rate of regrowth. A reduction in the rate of regrowth would allow the frequency of trimming to be reduced and savings in costs to be achieved.

Growth regulators, such as paclobutrazol (PP-333) (2RS, 3RS) -1-(4-chlorophenyl)-4, 4-dimethyl-2- (1,2, 4-triazol-1-yl) pentan-3-ol), flurprimidol (EL-500) (α -(1-methylethyl) - α - (4-trifluoromethoxy) phenyl) -5-pyrimidine-methanol) and uniconazole (XE-1019) ((E) -1- (4-chlorophenyl) -4, 4-dimethyl-2- (1, 2, 4 - triazol-1-yl) -1- penten-3-ol), have been reported to inhibit tree growth in a number of US and Canadian studies (see for example Miller, 1982; Hare, 1984; Aron et al, 1985; Arron, 1985, 1986; Sterrett, 1985, 1988; Green, 1986; Marini, 1986; Sterrett and Tworkoski, 1987; Gilliam et al., 1988; Watson, 1987; Watson and Johnston, 1987; Wood, 1988; Steffens, 1988). Of the various application methods used (eg trunk injection, bark banding, basal drench, soil injection, foliage application) trunk injection offers advantages over other methods in that the amount applied is precise, chemical contact with non-target plants is prevented and environmental residues are reduced (Sterrett, 1988).

Previous studies have shown that paclobutrazol, flurprimidol and uniconazole are translocated via the xylem into the foliage and shoots, while movement in the phloem to the roots (ie downwards) has not been detected (Sterrett, 1985, 1988; Wang et al, 1986, Sterrett & Tworkoski, 1987; Early and Martin, 1988). Application of these growth regulators to above ground plant parts (eg foliar spray, bark banding, trunk injection) would result in little or no product being translocated to the roots, while soil application (basal drench, soil injection) would result in the whole plant receiving some of the product. After growth regulators have been applied by trunk injection there is no direct release of the products into the environment. Following translocation into the foliage and young shoots paclobutrazol is thought to remain there (until the fall) (Richardson and Quinlan, 1986; Early and Martin, 1988). After leaf drop the growth regulator (and/or metabolites formed from them) enter the soil system surrounding the woody plant. Microbial activity in the leaf litter/soil will result in further breakdown of the growth regulator and/or metabolites. Such a mechanism of release of growth regulator into the

immediate plant environment is thought to be the primary path. There have been no reports of such growth regulators being found in leaf washes (rainfall), and since all these growth regulators are almost insoluble in water such a method of release is unlikely. The proportion of growth regulator, applied by trunk injection, that is released in foliage each fall has not been estimated.

The translocation studies described above (Sterrett, 1985, 1988; Wang et al, 1986; Sterrett and Tworkoski, 1987, Early and Martin, 1988) used young plant material and were short-term, terminating forty-two days or less after initiation. Questions such as what proportion of growth regulator (applied by trunk injection) is found in the foliage in the fall of the application year and later remain to be answered. The present study was designed to determine the effect uniconazole, applied by trunk injection, had on the growth of field-grown silver maple (Acer saccharinum L.) saplings in the eighteen months post application, the movement of the growth regulator in the saplings, and what concentration of applied growth regulator was present in the foliage over the same period. The presence of metabolites formed from uniconazole in the foliage collected in the fall was also investigated. The study was divided into two parts. In the first group of silver maple saplings, foliage samples were collected at regular intervals over the 1987 and 1988 growing seasons following trunk injection with ^{14}C -uniconazole in May 1987. Each fall the saplings were stripped of all the remaining foliage. ^{14}C activity in the foliage samples was then determined (foliage uptake experiment). A second group of saplings were trunk injected with ^{14}C -uniconazole and then whole saplings harvested (foliage, stem and roots) at various intervals. ^{14}C activity in all these organs of the saplings was determined (harvest experiment).

2.0 MATERIALS AND METHODS

2.1 Plant Material

Silver maple (Acer saccharinum L.) saplings, growing at Wesleyville G.S., were used in both the foliage uptake and the harvest experiments. The 4-year-old saplings were planted in a grid (4 x 4 m spacing) with eight saplings per row. Prior to initiation of the studies, the saplings were measured (overall height and stem diameter) and examined for health problems. Saplings with large or small stem diameters were excluded from the studies, as were saplings with dead limbs or bark damage around the base. The saplings were trimmed (29/4/87), with approximately one third of the crown being removed (height post trimming approximately 2.5 m, diameter at breast height (dbh) approximately 3 cm). Saplings in rows H, I, J, K, and L (with the exception of sapling H3) were used in the foliage uptake experiment, while saplings in rows A, B, C, D, E, F and G (plus sapling H3) were used in the harvest experiment. Row A was at the eastern end of the plot, with row L at the western end. In the foliage uptake experiment five saplings, selected at random, were injected with ^{14}C -uniconazole (see below) while five saplings were injected with the carrier, isopropyl alcohol. In the harvest experiment twelve saplings were injected with ^{14}C -uniconazole and six with isopropyl alcohol only.

2.2 Injection Procedure

Twenty-eight days after the saplings were trimmed, they were injected with ^{14}C -uniconazole. Holes were drilled in the sapling stem, about 40 cm above the soil surface, using an electric drill and a 0.2 cm diameter bit. The hole, at a slightly downward angle, was into the middle of the stem. Experimental trees were injected with 0.9 mL of uniconazole solution (containing 0.128 MBq ^{14}C -uniconazole (specific activity 7.096 MBq/g) in isopropyl alcohol) and then 0.5 mL isopropyl alcohol using a miniature pressure injector (see Sterrett and Creager, 1977). Control saplings were injected with 1.4 mL of isopropyl alcohol only. The injection holes were sealed with RTV (silicone) to prevent pathogen entry.

2.3 Sample Collection

In the foliage uptake experiment leaves (30 picked at random from each sapling) were collected from each of the five control and five experimental saplings immediately post-injection (day 0) and then 7, 17, 25, 35, 45, 65, 85, 101, 122, 133, 375, 416, 444, 472 and 497 days later. Samples 0 through 133 were collected in 1987 and 375 through 497 in 1988. On day 133 all the foliage that remained on the saplings was removed and the length of all the sprouts formed in the 1987 growing season measured. On day 497 the same procedure was conducted, with the 1988 sprouts being measured. Foliage samples were frozen and stored (-16°C) prior to determination of ^{14}C content (see below).

In the harvest experiment saplings injected with ^{14}C -uniconazole were sampled on day 0, 17, 134 and 500. On each occasion three saplings were dug up and cut into sections as outlined in Figure 1. Foliage, stem and root samples were stored frozen until ^{14}C content was determined. Three control saplings were harvested on day 0, 134, and 500, and divided up as in the experimental saplings. On days 134 and 500, prior to cutting up the saplings, the sprout growth (1987 and 1988 growing seasons, respectively) was measured in both the experimental and the control saplings.

2.4 Determination of ^{14}C Content of Foliage, Stem and Root Samples

Foliage, either the 30 leaf samples collected in the foliage uptake experiment or the foliage found on the sapling at the end of the growing season (foliage uptake and harvest experiments), was thawed and homogenized with distilled water using a Polytron or Vertis. The homogenate was frozen overnight (-16°C) and freeze dried. About 0.5 g (dry weight) of freeze dried powder was made into a pellet and oxidized (Packard Model 306 oxidizer) (Peterson 1969). The amount of $^{14}\text{CO}_2$ evolved after oxidation was determined by liquid scintillation spectrometry (Packard 300) using Permafluor V. Freeze dried foliage homogenates were retained for metabolite estimation (see below).

All stem samples (which were 15 cm long, see Figure 1), with the exception of the 15 cm stem section containing the injection point, were cut into three 5 cm long sections. The middle 5 cm section was chopped up into small pieces, freeze dried, ground up (coffee grinder) and approximately 0.4 g (dry weight) mixed with 0.1 g cellulose powder (to ensure an even burn in the oxidation step), made into a pellet and oxidized. The dry weight of the top and bottom

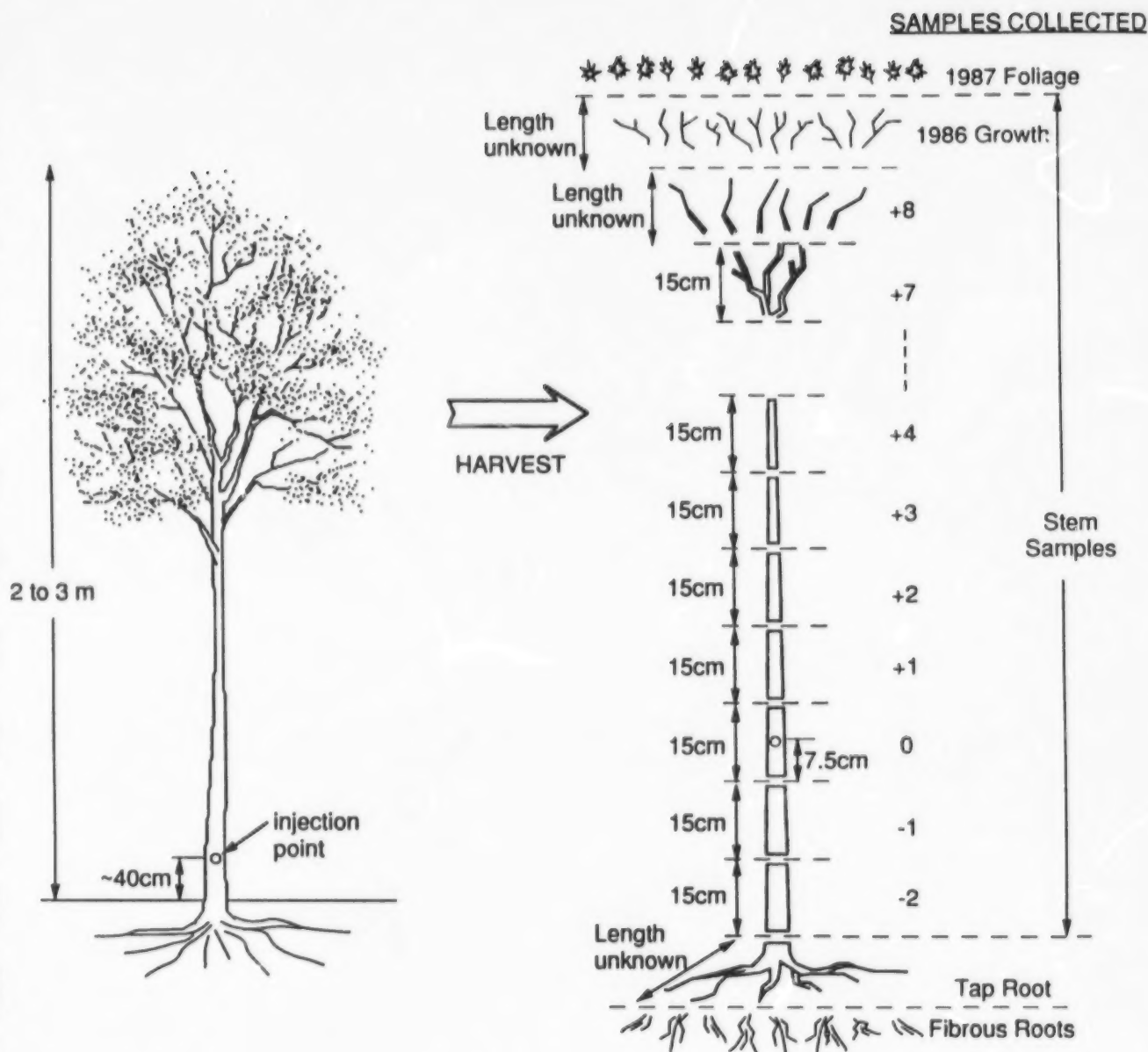


FIGURE 1

FOLIAGE, STEM AND ROOT TISSUE SAMPLES COLLECTED ON MAY 25TH, 1987 FROM SILVER MAPLE SAPLINGS IMMEDIATELY AFTER INJECTION WITH ^{14}C -UNICONAZOLE

Saplings harvested on May 25th, 1987 represent day 0 samples. Saplings were also harvested 17, 134 and 500 days after injection. In the day 134 saplings, a 1987 (stem) growth sample was collected in addition to the samples listed in the figure. In the day 500 saplings, 1987 (stem) growth and 1988 (stem) growth samples were collected in addition.

5 cm section was determined and the ^{14}C content of the whole 15 cm stem piece expressed on a per gram basis, or as the total number of Bq in the 15 cm section. This sub-sampling of the 15 cm stem sections was performed because of the time required to grind up whole stem sections. The +8 stem sections (see Figure 1) were of varied length. They were cut into 15 cm sections and treated as above. Sections of less than 15 cm were divided into 3 equal lengths and the middle part treated as above. The 1986 growth (and 1987 and 1988 growth) was either chopped up, freeze dried, ground up and oxidized, or if the sprout sections were long they were treated in the same way as the +8 stem sections described above. The stem section containing the injection point was divided into three 5 cm sections (top, middle and bottom) and each part chopped up, freeze dried, ground up and oxidized. The fibrous roots were removed from the rest of the root system, chopped up, freeze dried, ground up and oxidized. The tap root was sampled in the following manner. Tissue between 5 cm and 10 cm below the cut surface of the stump was removed and chopped up, etc. The dry weight of the remaining tap root tissue was determined.

2.5 Calculation of Uniconazole Concentration in Foliage and Sprout Samples

Estimates of the concentration of uniconazole, in foliage and sprout samples expressed either in terms of molarity or μg uniconazole per g dry weight, were calculated from the ^{14}C activity data, obtained as described above. In calculating the uniconazole concentration in terms of μg per g dry weight the following information was used; the total ^{14}C activity injected into the sapling (0.128 MBq) and the total weight of uniconazole injected (18.0 mg of unlabelled uniconazole and 0.0372 mg of labelled). In calculating the molar concentration of uniconazole the following additional information was used; the molecular weight of uniconazole (291.5), a dry weight to aqueous component ratio of 1.0:2.15 (reported for red maple foliage - Spencer, 1989) and a ratio of 1.0:0.7 for green wood (ie sprout, Hoadley, 1980). An additional assumption was that all the ^{14}C activity detected in the foliage and sprout samples was associated with uniconazole and not metabolites formed from uniconazole. The growth regulator properties of metabolites formed from uniconazole are not known and thus the assumption is valid (Sterrett, 1989). Metabolite formation was addressed in some foliage samples (see below).

2.6 Separation of ^{14}C -Uniconazole and Metabolites

In the determination of the proportion of ^{14}C present in uniconazole and metabolites, samples of the freeze dried 1987 foliage homogenates (2.5 g) were extracted (2x with 50 mL of 80% methanol (Sterrett, 1988). Distilled water (25 mL) was added and the extract evaporated to an aqueous solution. The pH was adjusted to 7.0 by adding distilled water (pH 12.0 adjusted with KOH), and the extract partitioned (3x) with 50 mL of methylene chloride. The pooled methylene chloride fractions were evaporated almost to dryness and the residue taken up in acetone. The extracts were then applied to TLC plates (Redi-Plate Silica Gel GF, 250 μm ; Fisher Scientific) and the chromatograms developed for 15 cm in chloroform: ethyl acetate: acetic acid (3:6:1 by volume) (Sterrett, 1988). Developed chromatograms were divided into 10 segments, the gel scraped off and the ^{14}C content quantified by liquid scintillation spectrometry using Picofluor 40. With 1988 foliage samples duplicate 5 g aliquots of freeze

dried foliage homogenate was extracted (2x) with 50 mL of 80% methanol as above. Following extraction with methylene chloride the fractions from the two extractions were pooled (in this way 10 g of each foliage sample was extracted rather than 2.5 g).

2.7 Determination of Leaf Colour

Foliage collected in the fall of 1987 and 1988 was termed senescing. In order that all the foliage could be obtained from a sapling it was necessary to strip the sapling in early October before all the foliage had changed colour. Waiting until later would have resulted in some leaves dropping off and this would not have allowed a mass balance calculation to be made.

From one sapling injected with ^{14}C -uniconazole, but not included in either the foliage uptake or the harvest experiments, leaves were collected on days 498 and 512. The sapling had responded non-uniformly to uniconazole treatment, with foliage on one major limb not showing the increased chlorophyll and more compact form shown in the rest of the sapling. Three 15 leaf samples were collected at random from the responsive limb (A) and the non-responsive limbs (B). The colour of each leaf was determined using a Munsell Colour Chart for Plant Tissues (Kollmorgen Corporation, Baltimore, Md.) and the number of leaves in the red, yellow/red, yellow and green/yellow categories recorded. A mean value was calculated from the three samples. ^{14}C activity in the samples was determined as for the 30 leaf samples described above.

3.0 RESULTS AND DISCUSSION

3.1 Effect of trunk injection of ^{14}C -uniconazole on the Growth of Silver maple saplings in the 1987 and 1988 Growing Seasons

In the 1988 growing season the saplings injected with uniconazole had a slightly different appearance from control saplings. Photographs of a control sapling (left) and an experimental sapling (right) are shown in Figure 2. The experimental sapling has a 'stacked' appearance (particularly the limb to the left of the photograph, with the leaves on the sprouts being closer together than in the control). 'Stacking' has been observed before in trees treated with paclobutrazol, flurprimidol and uniconazole. These growth regulators inhibit gibberellin biosynthesis and the reduced gibberellin level results in suppression of sprout internodal growth (Dalziel and Lawrence, 1984; Izumi et al 1985; Sterrett, 1985, 1988; Sterrett and Tworowski, 1987). The compressed form of the sprout leads to the bunched appearance of the foliage. In larger trees treated with growth regulators the canopy appears more open than in untreated trees. 'Stacking' was not observed in the present experiment in the 1987 growing season.

Sample leaves collected in 1988 from a sapling injected with uniconazole (to the right) and a control sapling (to the left) are shown in Figure 3. An increased chlorophyll pigmentation in the treated leaves is apparent, and this has been reported before with paclobutrazol, flurprimidol and uniconazole (Dalziel and Lawrence, 1984; Sterrett, 1985, 1988; Sterrett and Tworowski, 1987). An increase in chlorophyll content was not apparent in the 1987 growing season. The 'stacking' and increased pigmentation responses observed in 1988 did not occur in all the limbs of some of the experimental saplings.



FIGURE 2

APPEARANCE OF SILVER MAPLE SAPLINGS IN THE 1988 GROWING SEASON. (CONTROL SAPLING ON THE LEFT AND A ^{14}C -UNICONAZOLE-TREATED SAPLING ON THE RIGHT)



FIGURE 3

APPEARANCE OF FOLIAGE COLLECTED FROM SILVER MAPLE SAPLINGS IN THE 1988 GROWING SEASON. (TWO LEAVES FROM CONTROL SAPLING K1 ON THE LEFT, TWO LEAVES FROM ^{14}C -UNICONAZOLE-TREATED SAPLING K4 ON THE RIGHT)

Such non-uniform responses may have been the result of poor distribution of the growth regulator. The saplings were trunk injected at one point only. Uneven distribution of growth regulators between limbs has been reported before (Domir and Roberts, 1983). Uniconazole application to silver maple saplings had no effect on leaf dry weight (data included in Appendix A). Leaves were collected at random from the treated and control saplings at various times after injection and their ^{14}C activity content determined (see below). Over the course of the 1987 and 1988 growing seasons there was little change in dry weight on a per leaf basis in both the experimental and control saplings. Dalziel and Lawrence (1984) reported no effect of paclobutrazol on leaf dry weight but leaf area was reduced. Leaf thickness was increased and so dry weight per leaf tended to remain the same. Houde (1988) also reported a reduction of leaf area following treatment with paclobutrazol, flurprimidol and uniconazole. Leaf area was not measured in the present study.

The effects of trunk injection of uniconazole on the number of sprouts found on the silver maple saplings in the 1987 and 1988 growing seasons are shown in Table 1. In both the foliage uptake and the harvest experiments there was no significant difference in the number of sprouts (either all the sprouts or merely those above the injection point) found on the treated and control saplings. In both experiments sprouts were found below the injection point, around the base of the sapling. This is a common occurrence in young seedlings and saplings (Arron, 1985) but is rarer in more mature trees. In comparing the two growing seasons there was a trend towards more sprouts being formed in 1988 and this reflects an overall increase in sapling canopy size. In the foliage uptake experiment the mean number of sprouts per sapling was calculated including or excluding sapling J4 (Table 1). As will be seen later the foliage from sapling J4 contained very little ^{14}C activity (compared with the other four saplings in the treated group) and this suggested a problem with the injection procedure ie the sapling did not receive a complete dose of uniconazole.

The effects of uniconazole on the length of the sprouts formed in 1987 and 1988 are shown in Table 2. As in the previous table sapling J4 was included or excluded when mean sprout length was calculated. In the foliage uptake experiment there was no significant inhibition of sprout growth (both when all sprouts and those above the injection site only are considered) in either 1987 or 1988 as a result of uniconazole application. Sprouts below the injection site would not be expected to be influenced by the growth regulator as translocation of the product is upwards and not downwards from the injection point (Sterrett, 1988). For this reason any inhibition of sprout growth as a result of uniconazole application would be most apparent in sprouts found above the injection point. Sterrett (1988) reported inhibition of sprout growth by uniconazole in California privet, American sycamore, and yellow poplar. In the harvest experiment inhibition of sprout growth was observed in 1988 when sprouts above the injection point are considered (Table 2). The small sample number (3 saplings) in the harvest experiment and the lack of an effect in the foliage uptake experiment suggest that this result should be treated with caution. From the control data presented in Table 2 it appears that sprout growth in 1988 was less vigorous than in 1987, and this would be explained by the low rainfall in 1988. The soil at the experimental site is

Table 1

Effect of Trunk Injection of ^{14}C -uniconazole on the Mean Number of Sprouts formed on Silver Maple Saplings in the 1987 and 1988 Growing Seasons

Experiment	Treatment	Growing Season	Number of Sprouts per Sapling	
			All Sprouts	Above Injection Site Below Injection Site
----- (mean $\pm$ s.d.) ⁺ -----				
Foliage	Control	1987	74 $\pm$ 24	69 $\pm$ 24
Foliage	Uniconazole	1987	82 $\pm$ 35	68 $\pm$ 28
Foliage	Uniconazole*	1987	89 $\pm$ 37	75 $\pm$ 25
Foliage	Control	1988	150 $\pm$ 91	147 $\pm$ 91
Foliage	Uniconazole	1988	114 $\pm$ 50	108 $\pm$ 47
Foliage	Uniconazole*	1988	130 $\pm$ 41	123 $\pm$ 39

Harvest	Control	1987	92 $\pm$ 43	77 $\pm$ 31
Harvest	Uniconazole	1987	96 $\pm$ 21	92 $\pm$ 20
Harvest	Control	1988	185 $\pm$ 94	179 $\pm$ 100
Harvest	Uniconazole	1988	216 $\pm$ 48	201 $\pm$ 48

				5 $\pm$ 6
				15 $\pm$ 12
				14 $\pm$ 13
				3 $\pm$ 3
				6 $\pm$ 5
				7 $\pm$ 5

				15 $\pm$ 13
				4 $\pm$ 6
				6 $\pm$ 6
				15 $\pm$ 10

+ In the foliage experiment the mean was calculated from five saplings, in the harvest experiment three saplings were used.

* Sapling J4 excluded from the data set (n=4).

(C32)

Table 2

Effect of Trunk Injection of ^{14}C -uniconazole on the Length
of Sprouts formed on Silver Maple Saplings in the 1987 and 1988
Growing Seasons - Mean Values

Experiment	Treatment	Growing Season	Sprout Length (cm)**		
			All Sprouts	Above Injection Site	Below Injection Site
------(mean $\pm$ s.d.) +-----					
Foliage	Control	1987	5.1 $\pm$ 1.3	4.5 $\pm$ 1.1	11.3 $\pm$ 3.9
Foliage	Uniconazole	1987	6.4 $\pm$ 2.0	5.4 $\pm$ 2.4	11.9 $\pm$ 1.8
Foliage	Uniconazole*	1987	6.0 $\pm$ 2.0	5.0 $\pm$ 2.6	12.2 $\pm$ 2.0
Foliage	Control	1988	3.8 $\pm$ 0.7	3.6 $\pm$ 0.8	13.8 $\pm$ 6.5
Foliage	Uniconazole	1988	4.3 $\pm$ 0.8	3.9 $\pm$ 1.2	9.5 $\pm$ 3.3
Foliage	Uniconazole*	1988	3.9 $\pm$ 0.3	3.5 $\pm$ 0.7	10.8 $\pm$ 2.3

Harvest	Control	1987	8.2 $\pm$ 4.0	8.0 $\pm$ 4.1	6.6 $\pm$ 3.4
Harvest	Uniconazole	1987	5.9 $\pm$ 2.5	5.8 $\pm$ 2.3	11.1 $\pm$ 1.3
Harvest	Control	1988	4.4 $\pm$ 0.8	4.1 $\pm$ 1.1	11.0 $\pm$ 1.9
Harvest	Uniconazole	1988	2.8 $\pm$ 0.8	1.9 $\pm$ 0.8	11.6 $\pm$ 2.7

* Sapling J4 excluded from the data set

** Sprouts were measured to the nearest 0.5 cm, sprouts with a length of 1 cm or less were recorded as being 1 cm in length

+ Mean sprout length was calculated for each sapling (five saplings in the foliage experiment and three in the harvest), and then a mean (per sapling basis) calculated from these five (or three) values

(C32)

extremely sandy and does not have good water holding properties. Growth of sprouts below the injection point appeared to exceed that of the remaining sprouts on the saplings, and this agrees with previous results from studies of growth regulator effects on silver maple seedlings (Arron, 1985).

The 'stacking' of foliage observed in the uniconazole treated saplings would suggest internode compression and thus the apparent failure to observe inhibition of sprout growth in the foliage uptake experiment was confusing. In previous studies it has been found that mean sprout length is not a useful measure of growth (see Camacho and Arron, 1988) while median sprout length is. Median sprout lengths (for sprouts formed above the injection point) from the present experiments are given in Table 3. The lower level of growth in the 1988 growing season compared to 1987 was again apparent. In 1988 an inhibition of sprout growth was observed in the harvest experiment and in the foliage uptake experiment (but only if sapling J4 was excluded) (Mann-Whitney non-parametric test). However, problems are still evident with the data. For example in the harvest experiment all three saplings in the experimental and control groups had a median sprout length of 1.0 and 1.5 cm respectively. In taking measurements of sprout length, those sprouts of 1 cm and less in length were recorded as being 1 cm long, and sprouts were measured to the nearest 0.5 cm. Thus the data collected were discontinuous, being 1.0, 1.5, 2.0 cm, etc. The preponderance of extremely short sprouts in both experimental and control saplings meant the short-comings of this method of data collection were exposed. Given that enough uniconazole was injected into the saplings to have an effect on sprout growth, the problems of data analysis and interpretation and the inability to demonstrate a large-scale inhibition of sprout growth described above, were encountered when every sprout on a sapling was measured. In more mature trees it is not possible to measure every sprout. Other methods of estimating tree growth (biomass increase, increase in crown size) which use photographs and video pictures (to permit image analysis) might be useful in growth regulator studies.

3.2 ^{14}C Activity in the Foliage of Silver Maple Saplings in 1987 and 1988 following Trunk Injection with ^{14}C -uniconazole (Foliage Uptake Experiment)

Changes in ^{14}C activity in 30 leaf samples collected in the 1987 and 1988 growing seasons are shown in Figure 4. ^{14}C activity is given in Bq/g dry weight and similar curves were observed when the results were expressed on a per leaf basis since, as has been discussed before, there was little change in leaf dry weight over the course of the experiment. Following injection of the ^{14}C -uniconazole into the saplings there was an increase in the concentration of ^{14}C detected in the foliage. In the spring of 1988 the concentration of ^{14}C was extremely low and with time it increased until the fall. The maximum concentration detected in 1988 was approximately one third the level reached in 1987. The similarities in the curves for 1987 and 1988 can be explained when one considers that the foliage formed during each growing season constitutes a new sink for deposition of material carried in the transpiration stream. The concentration of ^{14}C activity in the foliage will increase, assuming none is lost from the foliage, as a function of the amount of water passing in and out of the foliage in the transpiration stream. The data presented in Figure 4 are for four saplings only. Sapling J4, for which the maximum ^{14}C activity detected was 4.16 Bq/g dry weight (compared to the

TABLE 3

Effect of Trunk Injection of ^{14}C -Uniconazole on the
Length of Sprouts Formed Above the Injection
Site on Silver Maple Saplings in the 1987 and 1988
Growing Seasons - Median Values

Experiment	Treatment	Sapling	Median Sprout Length (cm)**	
			1987	1988
Foliage	Control	K1	2.00	2.00
Foliage	Control	J1	1.50	1.75
Foliage	Control	J7	2.75	1.00
Foliage	Control	H2	2.75	2.00
Foliage	Control	I5	2.00	1.50
Foliage	Uniconazole	K4	2.50	1.00
Foliage	Uniconazole	J3	1.50	1.00
Foliage	Uniconazole	I6	2.00	1.00
Foliage	Uniconazole	I3	1.00	1.00
Foliage	Uniconazole	J4	3.75+	2.50+
Harvest	Control	F6, D7*	1.0	1.5
Harvest	Control	E8, B8	3.0	1.5
Harvest	Control	C3, A4	5.0	1.5
Harvest	Uniconazole	C6, F1	1.0	1.0
Harvest	Uniconazole	F5, B3	2.0	1.0
Harvest	Uniconazole	C4, B5	2.0	1.0

* Second sapling harvested in 1988

** Sprouts were measured as described in Table 2.

+ Foliage from sapling J4 contained low levels of ^{14}C activity in both 1987 and 1988 (perhaps as a result of loss on injection)

(C32)

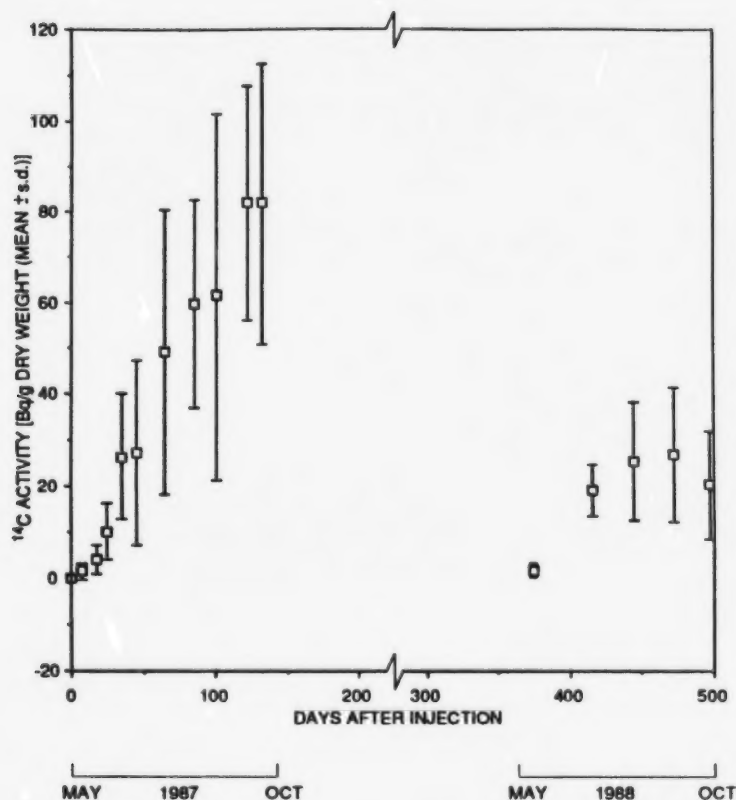


FIGURE 4

INCREASE IN ¹⁴C ACTIVITY IN FOLIAGE COLLECTED IN 1987 AND 1988 FROM SILVER MAPLE SAPLINGS, TRUNK INJECTED WITH ¹⁴C-UNICONAZOLE ON MAY 25, 1987

Mean values shown are for four saplings (J4 excluded). ¹⁴C activity values for J4 did not exceed 4.16 Bq/g dry weight in 1987 and 0.79 Bq/g in 1988. Rationale for J4 exclusion provided in text. Mean values for control saplings did not exceed 0.021 Bq/g in 1987 and 1988 (days 375 and 497 sampled only)

TABLE 4

Proportion of ^{14}C Activity Found in the Foliage of Silver Maple
 Saplings trunk Injected with ^{14}C -Uniconazole (Foliage Uptake Experiment)

Treatment	Year	Total Number of Leaves Collected per Sapling (mean $\pm$ s.d.)	Total Dry Weight of Leaves Collected per Sapling (g) (mean $\pm$ s.d.)	Total ^{14}C Activity in Leaves, per Sapling (Bq) (mean $\pm$ s.d.)	^{14}C Activity in Leaves Collected as Function of Amount Injected (%) (mean $\pm$ s.d.)
Control	1987	822 $\pm$ 180	150 $\pm$ 59	+	-
Control	1988	1140 $\pm$ 433	145 $\pm$ 66	+	-
Uniconazole	1987	760 $\pm$ 175	159 $\pm$ 43	7392 $\pm$ 4374	5.8 $\pm$ 3.4
Uniconazole	1988	906 $\pm$ 340	145 $\pm$ 47	2380 $\pm$ 1470	1.9 $\pm$ 1.2
Uniconazole*	1987	800 $\pm$ 175	173 $\pm$ 35	9193 $\pm$ 1972	7.2 $\pm$ 1.5
Uniconazole*	1988	980 $\pm$ 344	156 $\pm$ 47	2972 $\pm$ 734	2.3 $\pm$ 0.6

* Sapling J4 excluded from mean calculation

+ Four of five saplings had a negative ^{14}C activity in foliage - see Appendix B

- No uniconazole injected (control saplings)

(C32)

maximum mean value for the four remaining saplings of about 80 Bq/g), was excluded from the data set. Inclusion of the data for J4 gave a lower mean value but the overall shape of the curve was the same. The lack of appearance of ^{14}C activity in the foliage of sapling J4 was probably the result of severe leakage at the time of injection.

In previous translocation studies Richardson and Quinlan (1986) reported no movement of paclobutrazol out of older apple leaves and little movement out of younger leaves. Early and Martin (1988) also reported no movement of paclobutrazol out of peach leaves, but the experiment was only conducted for 9 days. Wang et al (1986) found a continuous increase in the amount of paclobutrazol present in apple leaves over 42 days (paclobutrazol was applied to the roots, plants grown hydroponically).

The lower concentrations of ^{14}C activity detected in foliage in the second year in the present experiment may be explained by the lower rainfall in the second year, which would have resulted in less water being lost through transpiration. Various other factors will be important in determining the concentration of ^{14}C activity in the foliage. The size of the foliage sink should have increased from 1987 to 1988 as the saplings developed. A larger sink, in terms of total dry weight of tissue, would mean a reduction in the concentration of ^{14}C activity. Data on both leaf number and total dry weight change from 1987 to 1988 are inconclusive (see Table 4). Leaf number changed from 822 ± 180 per sapling in the control in 1987 to 1140 ± 433 in 1988 while in the uniconazole group it was 760 ± 175 and 906 ± 340 respectively. Total leaf dry weight was 150 ± 59 g and 145 ± 66 g in the control and 159 ± 43 g and 145 ± 47 g in the uniconazole. However, none of these changes were significant, and an explanation of the lack of a dry weight increase might be the low rainfall in 1988. A similar lack of an effect on leaf number and dry weight was apparent in the harvest experiment (see Table 5). Wood biomass would have increased from 1987 to 1988 and thus this sink would have been larger also.

The size of the reservoir of ^{14}C labelled material in the sapling would be reduced from year to year as some of it is lost in the foliage in the fall and perhaps as a result of callus tissue forming around the injection site and 'trapping' the ^{14}C -uniconazole. A smaller reservoir would lead to a lower concentration of ^{14}C activity in the foliage (assuming an equal amount of water available to translocate the material from year to year). An attempt was made to estimate the reduction in the reservoir size by determining how much ^{14}C -labelled material was lost in foliage dropped in the fall. The total amount of ^{14}C activity detected in all the foliage collected in either 1987 or 1988 was expressed as a function of the amount of ^{14}C -uniconazole injected into each sapling at the initiation of the experiment (see Table 4). In the four saplings treated with uniconazole $7.2 \pm 1.5\%$ and $2.3 \pm 0.6\%$ of the total ^{14}C -uniconazole injected was present in the foliage in 1987 and 1988 respectively. As discussed above, sapling J4 was excluded from the data set. (Data from the individual saplings are given in Appendix B.) The reduction in the size of the reservoir, attributable to loss in foliage, was approximately 9.5% over two growing seasons. Results from long-term growth regulator studies with paclobutrazol have indicated that the product is effective in inhibiting woody growth over two or three seasons, and this would suggest that little product is released from the woody plant in foliage (or as root and

leaf exudates) or the product is broken down to give metabolites with the same or similar growth-retarding properties as the product itself. In estimating the amount of ^{14}C activity in the foliage as a percentage of that injected the assumption was made that removal of foliage samples every few weeks had no effect on the total number of leaves formed over the entire growing season. Foliage removal might stimulate or inhibit the initiation of new leaves. Removal of leaves early in the season might lead to an underestimation of the final foliage content of ^{14}C activity, since leaving the foliage on the sapling for the whole season would result in higher ^{14}C activity, assuming no change in foliage numbers. This criticism was addressed in the harvest experiment, where the saplings were injected and left until the fall when the whole plant was harvested at once. Results from this experiment are given in Table 5. At the end of the 1987 growing season the ^{14}C activity in foliage expressed as a percentage of that injected was $7.1 \pm 1.7\%$, and at the end of the 1988 growing season was $2.3 \pm 0.6\%$. These mean values compare very well with those from the foliage uptake experiment (Table 4) and suggest that periodic removal of leaves had little effect if any on the proportion of ^{14}C -labelled material translocated into the foliage over a growing season. (The data for the individual saplings in the harvest experiment are given in Appendix C.)

The results of ^{14}C activity found in the foliage and described above are consistent with some of the small number of studies on uniconazole, paclobutrazol and flurprimidol translocation published to date. Sterrett (1988) found only 2% of the ^{14}C activity detected in young apple trees, 28 days after trunk injection with ^{14}C -uniconazole, was in young shoots (these consisted of foliage plus young sprouts). In experiments with flurprimidol and paclobutrazol conducted in the same way this author reported 10% of ^{14}C activity in young shoots (35 days after trunk injection of ^{14}C -flurprimidol) and 23% in young shoots (27 days after trunk injection of ^{14}C -paclobutrazol) (Sterrett, 1985; Sterrett and Tworowski, 1987). Wang et al (1986) on the other hand reported that 53% of the paclobutrazol present in apple seedlings was in the foliage 42 days after beginning the continuous supply of the growth regulator through the roots via nutrient solution. In similar experiments conducted by Early and Martin (1988) with peach seedlings, again grown in nutrient solution and paclobutrazol, 29% of the ^{14}C activity found in the seedlings 9 days after exposure (continuous) was present in the foliage. The solubility in water of the three growth regulators, uniconazole, paclobutrazol and flurprimidol, has been reported to be low. There may be few differences between application of growth regulators via the roots (plants grown hydroponically) and by trunk injection since in the latter the products precipitate out after injection and then act as a large reservoir within the stem, to be translocated to the upper plant in a continuous fashion. This is described in more detail below.

When the remaining foliage was removed from the saplings in the present foliage uptake experiment in the fall of 1987 and 1988 the leaves had just begun to turn. Leaving the foliage on the saplings longer might have resulted in loss of some leaves (turning and abscission in the saplings is non-uniform) and a consequent inability to determine accurately the amount of ^{14}C present in the foliage. Early removal of foliage might lead to overestimating ^{14}C activity, as growth regulator might be withdrawn at the same time as chlorophyll is broken down and the products translocated out of the leaves. A

TABLE 5

Proportion of ^{14}C Activity Found in the Foliage of Silver Maple
Sampling trunk injected with ^{14}C -Uniconazole (Harvest Experiment)

Treatment	Days After Injection	Total Number of Leaves Collected per Sapling (mean $\pm$ s.d.)	Dry Weight of Leaves per Sapling (g) (mean $\pm$ s.d.)	Total ^{14}C Activity per Sapling (Bq) (mean $\pm$ s.d.)	^{14}C Activity in Leaves Collected as Function of Amount Injected (a) (mean $\pm$ s.d.)
Control	0	425 $\pm$ 87	55 $\pm$ 8	*	-
Control	134	883 $\pm$ 292	272 $\pm$ 29	*	-
Uniconazole	0	476 $\pm$ 82	73 $\pm$ 31	+	0.0 $\pm$ 0.0
Uniconazole	17	559 $\pm$ 98	103 $\pm$ 30	1874 $\pm$ 934	1.5 $\pm$ 0.7
Uniconazole	134	876 $\pm$ 187	190 $\pm$ 34	9301 $\pm$ 2153	7.1 $\pm$ 1.7
Uniconazole	500	1317 $\pm$ 154	175 $\pm$ 87	2932 $\pm$ 743	2.3 $\pm$ 0.6

* ^{14}C activity found in control samples day 0 and 134, and in uniconazole sample day 0 was extremely low, some values were negative (for complete list see Appendix C)

- No uniconazole injected (i.e. control saplings)

+ Values for the three saplings were 0, 6 and -5 Bq giving a mean of 0.3 ± 5.5

(C32)

secondary experiment was conducted in 1988 to address this. A sapling, not included in either the foliage uptake or the harvest experiments, which had been injected with ^{14}C -uniconazole in May 1987 was used. The response of the sapling to uniconazole was non-uniform, with one major limb (Limb A) showing stacking and an increase in chlorophyll while the remainder of the sapling showed no response (Limb B). Three 15 leaf samples were removed from both Limb A and Limb B on day 498 and day 512. Leaf colour had changed in that time from predominantly green/yellow on both Limb A and B to yellow (see Table 6). The Munsell Colour Chart used to estimate leaf colour can break down the green/yellow classification into subdivisions on the basis of value and chroma, however this was not necessary in this experiment. While leaf colour had changed between 498 and 512 days there was no change in the ^{14}C activity found in the foliage from Limb A and perhaps an increase in Limb B (Table 6). The ^{14}C activity found in foliage from Limb A was many times higher than that found in foliage from Limb B (which did not show any morphological response to the growth regulator). These results suggest that growth regulator present in foliage is not mobilized and moved back into the woody plant as the foliage turns in the fall. Previous studies with paclobutrazol have indicated little or no movement of the growth regulator out of foliage (Richardson and Quinlan, 1986; Early and Martin, 1988).

3.3 ^{14}C Activity in the Stem, Roots and Foliage of Silver Maple Saplings in 1987 and 1988 following Trunk Injection with ^{14}C -Uniconazole (Harvest Experiment)

Silver maple saplings trunk injected with ^{14}C -uniconazole on May 25th, 1987 were harvested on the day of injection (day 0), and then 17, 134 and 500 days later. On each occasion three experimental saplings were harvested and divided up into stem, root and foliage samples (see Figure 1). Control saplings, injected with isopropyl alcohol only, were harvested on day 0 and 134.

Examination of the various stem sections revealed staining of the wood in saplings harvested at 134 and 500 days. An area of light brown stain, covering approximately one third of the area of the stem in cross section, extended from the injection point upwards for a distance of approximately 30 cm and a similar distance downwards (see Table 7). The stained area was confined to one side of the stem and with distance from the injection point the area was reduced. Whether the stained area remained on the same side of the stem with distance from the injection point was not determined. Stained wood was observed in all saplings harvested at 134 and 500 days but in only one control sapling (134 days). Control saplings harvested at 500 days were not examined. The fact that stained wood was observed both above and below the injection point suggests that it was the injection process that forced the staining agent into such a volume of wood, rather than the transpiration stream distributing the agent. The volume of stained wood in the saplings was quite large, between 8 and 16 cc (calculated assuming two cones, one pointing upwards and one down, each with a radius of 0.5 cm and a height of 30 cm). Only 1.5 mL of solution was injected, but immediate dilution would have taken place upon entry into the aqueous stem interior. Uniconazole is practically insoluble in water (15 ppm) (Anonymous, 1986) and so will precipitate out instantly once injected into the stem. The almost complete lack of stained wood in the control saplings suggest that isopropyl alcohol was not the cause of the stain but that uniconazole was. The experimental saplings were

TABLE 6

Change of Colour and ^{14}C Activity in Foliage Collected
498 and 512 Days after Injection of a Silver Maple Sapling
With ^{14}C -Uniconazole

Days after Injection	Foliage from Limb A				Foliage from Limb B				
	R	Leaf Colour+		¹⁴ C Content* - Bq/leaf -	R	Leaf Colour+		¹⁴ C Content* - Bq/Leaf -	
		YR	Y GY			YR	Y GY		
498	-	-	0.6	14.5	2.24 ± 0.60	-	-	15.0	0.28 ± 0.11
512	0.9	4.6	9.6	-	2.42 ± 0.52	0.3	1.0 12.0	1.7	0.47 ± 0.08

* Mean value (of 3) quoted $\pm$ s.d.

+ Leaf colour: Key R = red; YR = yellow/red; Y = yellow; GY = green/yellow. Values quoted are rounded up, and represent the mean (n=3) number of leaves in that particular colour group. Each sample contained 15 leaves.

(C32)

injected with uniconazole (^{14}C -labelled and non-labelled technical grade material) and isopropyl alcohol only, no surfactants were included (as might be found in the formulated product). This observation contradicts the findings of Houde (1988) who reported that isopropyl alcohol causes the staining of wood in extensive studies in Quebec. Methanol, the carrier used with the formulated product of paclobutrazol, caused more extensive staining than isopropyl alcohol (Houde, 1988). Support for the conclusion that uniconazole causes the staining of wood is provided by the ^{14}C activity distribution data given below.

^{14}C activity detected in the various stem, root and foliage samples collected from saplings trunk injected with ^{14}C -uniconazole and harvested 0, 17, 134 and 500 days is illustrated in Figure 5. For each of the samples collected the total ^{14}C activity was determined (specific activity [Bq/g] x total dry weight) and this value expressed as a function (percentage) of the total ^{14}C activity injected into the sample in 1987. On day 0 most of the ^{14}C activity was confined to stem sample 0 and the samples immediately above and below it (+1 and -1). The injection process had forced the growth regulator into these stem sections, as the saplings were harvested immediately after being injected. As would be expected no ^{14}C activity was detected in the foliage. The shape of the ^{14}C activity distribution, with higher ^{14}C activity in stem samples +1 and -1 than in sample 0 (seen in the 17, 134 and 500 day saplings) may have been the result of the injection method used, with the 0.9 mL of ^{14}C -labelled material being followed by 0.5 mL of isopropyl alcohol. The alcohol may have forced the ^{14}C -labelled material upwards and downwards from the injection point, and the alcohol would have been left in stem sample 0. The distribution of ^{14}C activity in the stem is much like the distribution of stained wood (see Table 7) and this supports the conclusion that staining was a response to the growth regulator and not the alcohol.

With time ^{14}C activity was distributed more widely in the saplings, with some being found in the upper stem samples and the foliage at 17 days, and then more in the foliage at 134 days (Figure 5). The percentage ^{14}C activity recovered in the foliage has been discussed previously (see Table 5). By 500 days the distribution was almost unchanged, with some reduction in the percentage ^{14}C activity detected in the foliage (day 134 was 1987 foliage and day 500 was 1988). Over the time course little ^{14}C activity was detected in the tap root segments, with the highest level (mean of 0.75%) being found at 500 days. Uniconazole does not move downwards following trunk injection in apple (Sterrett, 1988) and the present study agrees with this finding. With time there was no evidence of a front of ^{14}C activity moving up the saplings in response to a continuous (day time) movement of water upwards. The stem samples +1, 0 and -1 continued to contain most of the ^{14}C activity. Similar results have been reported by Sterrett and coworkers. In apple trees injected with ^{14}C -flurprimidol 80% of the ^{14}C activity was still located around the injection point 35 days after application of the growth regulator (Sterrett and Tworkoski, 1987). With paclobutrazol 58% of the ^{14}C activity was found around the injection site 27 days after application (same experimental design as in Sterrett and Tworkoski, 1987) (Sterrett, 1985). Similar results were obtained with uniconazole (Sterrett, 1988) where although no percentage was quoted, it was apparent from the data given in a figure that about 90% of the ^{14}C activity present after 28 days was around the injection site (again same experimental details as in Sterrett and Tworkoski, 1987). The distribution of

TABLE 7

Stained Wood Found in Silver Maple Saplings Harvested 134 and 500 Days After Injection With ^{14}C -Uniconazole or Isopropyl Alcohol (Control)

Stem/Root Sample ** (See Figure 1)	Days After Injection								
	+ 134			+ 134			+ 500*		
	Control			Uniconazole			Uniconazole		
	Sapling			Sapling			Sapling		
	1	2	3	1	2	3	1	2	3
+5									
+4									
+3									
+2									
+1									
0 Top (5 cm)									
0 Middle (5 cm)									
0 Bottom (5 cm)									
-1									
-2									
Tap root									

* Control saplings from 500 days after injection were not examined.

**All stem samples were 15 cm in length unless indicated.

I Stained wood observed in stem cross section. At the injection point the area of stain was approximately one third of total stem cross section area, and this was reduced the further above or below the injection point the stain was found.

(C32)

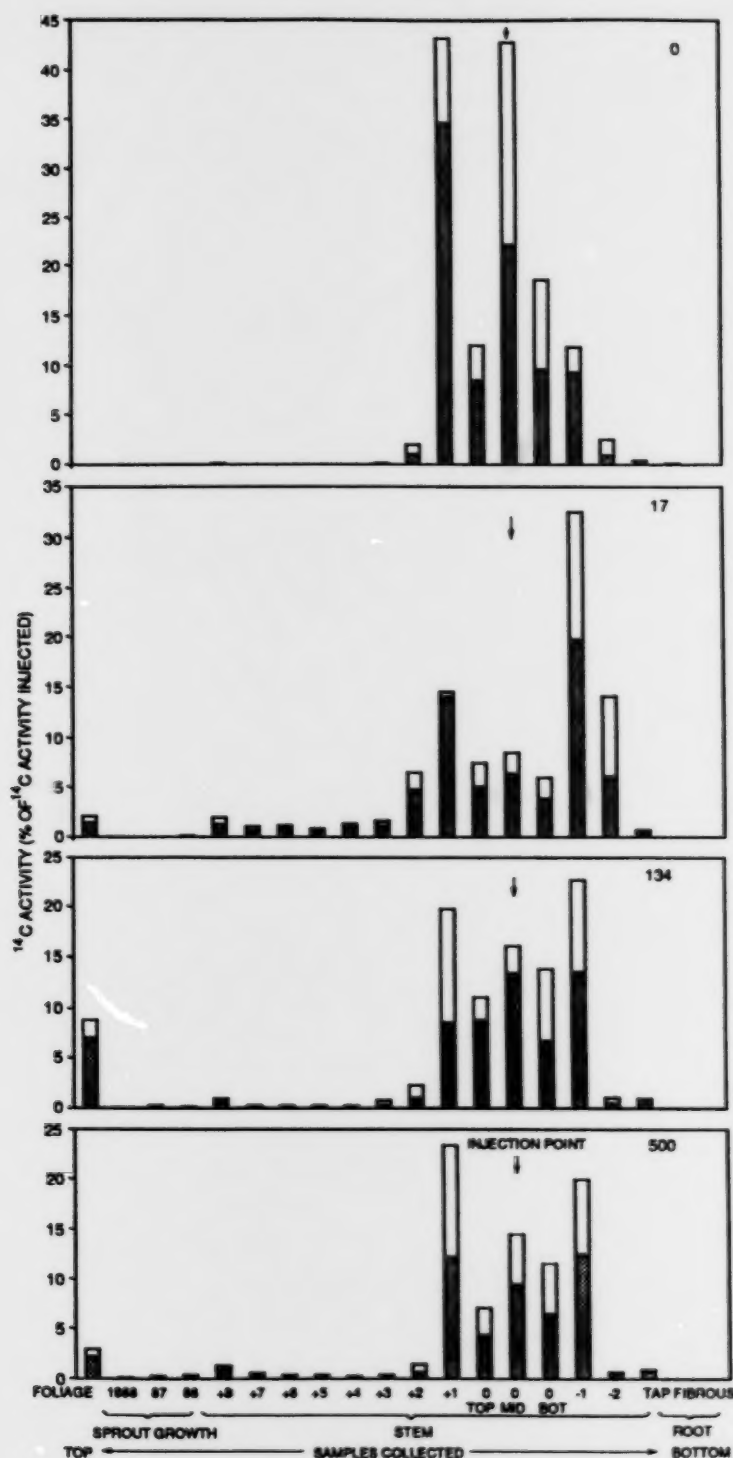


FIGURE 5
DISTRIBUTION OF ^{14}C ACTIVITY IN STEM, ROOT AND FOLIAGE SAMPLES FROM SILVER MAPLE SAPLINGS FOLLOWING TRUNK INJECTION WITH ^{14}C -UNICONAZOLE ON MAY 25, 1987

Stem, root and foliage samples were collected (see Figure 1) from silver maple saplings harvested 0, 17, 134 and 500 days after trunk injection with ^{14}C -uniconazole. Shaded bar represents mean value, and open bar mean + s.d. (n = 3)

^{14}C activity seen in the present study, with little activity being found in the stem between the wood around the injection point and the foliage, agrees with results from other studies. Early and Martin (1988) who grew peach seedlings in a nutrient solution containing labelled paclobutrazol found equal amounts of ^{14}C activity in the roots and leaves, and very low levels in the stem. Wang et al (1986), who grew apple seedlings in the same way, found after 42 days that 53% of the ^{14}C activity was in the foliage and 26% and 21% in the stem and roots respectively.

A close examination of the data in Figure 5 suggests that the retrieval of ^{14}C activity (i.e. total ^{14}C activity detected as a function of total ^{14}C activity injected) declined with time. The maximum percentage of ^{14}C activity in any part of the sapling was 34.6% at day 0, and this declined to 19.8, 13.6 and 12.5% by days 17, 134 and 500 respectively. A similar decline is seen in the percentage of ^{14}C activity in stem sample 0 (data for top, middle and bottom combined - Figure 5), from 40.2 to 20.3% (day 0 and day 500 respectively) and in stem samples +1, 0 and -1 combined, 84.1 to 45.0% (see Table 8). Retrieval of ^{14}C activity for whole saplings declined from 86.7% to 52.7% over the same period. A similar decline in the percentage of ^{14}C activity recovered was reported for both flurprimidol and paclobutrazol (Sterrett, 1985; Sterrett and Tworowski, 1987). When the drop in the retrieval of ^{14}C activity is taken into account and the percentages of ^{14}C activity in stem sample 0 or +1, 0 and -1 combined are calculated as a function of ^{14}C retrieved (and not injected), then a decline is much less evident (see Table 8). For stem sample 0 at day 0 the percentage of ^{14}C activity was 46.0%, and at day 500 it was 39.1%. Similarly for stem samples +1, 0 and -1 combined, values were 97.0 and 83.8% respectively. Expressing ^{14}C activity in foliage in the same way gave values of 11.3 and 4.6% at days 134 and 500, respectively (7.1 and 2.3% of ^{14}C activity injected). This apparent decline in ^{14}C activity retrieval with time has no ready explanation. Since most of the ^{14}C activity remained confined to the injection site, it should have been possible to estimate this activity quite easily. A wider distribution leading to uniformly low levels throughout the sapling might have introduced greater errors (usually leading to underestimation) because of an inability to measure low levels accurately. Some ^{14}C activity may have been lost or released from the saplings. At the end of 1987 about 7% of the ^{14}C activity injected into each sapling was lost in the form of dropped foliage. This would have meant a reduction in the total ^{14}C activity remaining to 93% by the beginning of 1988 - hardly a significant reduction. ^{14}C activity could have been lost in the form of leaf runoff or exudate, root exudates, or in the form of $^{14}\text{CO}_2$ after complete metabolism of the growth regulator. The latter is most unlikely given the long-term inhibition of growth caused by uniconazole (Anonymous, 1986). There have been no reports of growth regulators being released from plants in leaf or root exudates, or in leaf runoff.

The ^{14}C activity distribution data, discussed above, are in the form of a mass balance, with the ^{14}C activity found in the various foliage, stem, and root samples being expressed as a function of either the total activity injected into the sapling or the total activity retrieved in all the samples harvested from a sapling. Such data do not provide information on the concentration of ^{14}C activity in any particular sample. The data for the 500 day saplings shown in Figure 5 are expressed on a Bq/g dry weight basis in Figure 6. ^{14}C activity around the injection site was extremely high and very low in all

TABLE 8

Retrieval of ^{14}C Activity from Harvested Whole Silver Maple Saplings
Following Trunk Injection with ^{14}C -Uniconazole and Proportion
of ^{14}C Activity in Foliage and Selected Stem Samples

Days after Injection	Retrieval of ^{14}C Activity in Whole Saplings	-----Stem Samples ⁺ -----			Foliage
		0	+1	0 -1	
	- % of ^{14}C Injected* -	-----% of ^{14}C Injected*-----			
0	86.7 $\pm$ 7.1	40.2 $\pm$ 14.2 [46.0]++	84.1 $\pm$ 8.8 [97.0]	0.0 $\pm$ 0.0 [0.0]	
17	67.6 $\pm$ 25.6	15.2 $\pm$ 5.1 [22.8]	49.1 $\pm$ 18.0 [72.9]	1.5 $\pm$ 0.7 [2.5]	
134	62.6 $\pm$ 5.3	29.0 $\pm$ 8.1 [46.2]	51.1 $\pm$ 4.5 [81.6]	7.1 $\pm$ 1.7 [11.3]	
500	52.7 $\pm$ 17.8	20.3 $\pm$ 6.2 [39.1]	45.0 $\pm$ 18.7 [83.8]	2.3 $\pm$ 0.6 [4.6]	

* Values given are mean $\pm$ s.d. (n=3)

+ For location of stem samples in saplings see Figure 1

++ Value in bracket represents percentage ^{14}C activity in particular foliage or stem sample(s) as function of total ^{14}C activity retrieved (mean value, n=3)

(C32)

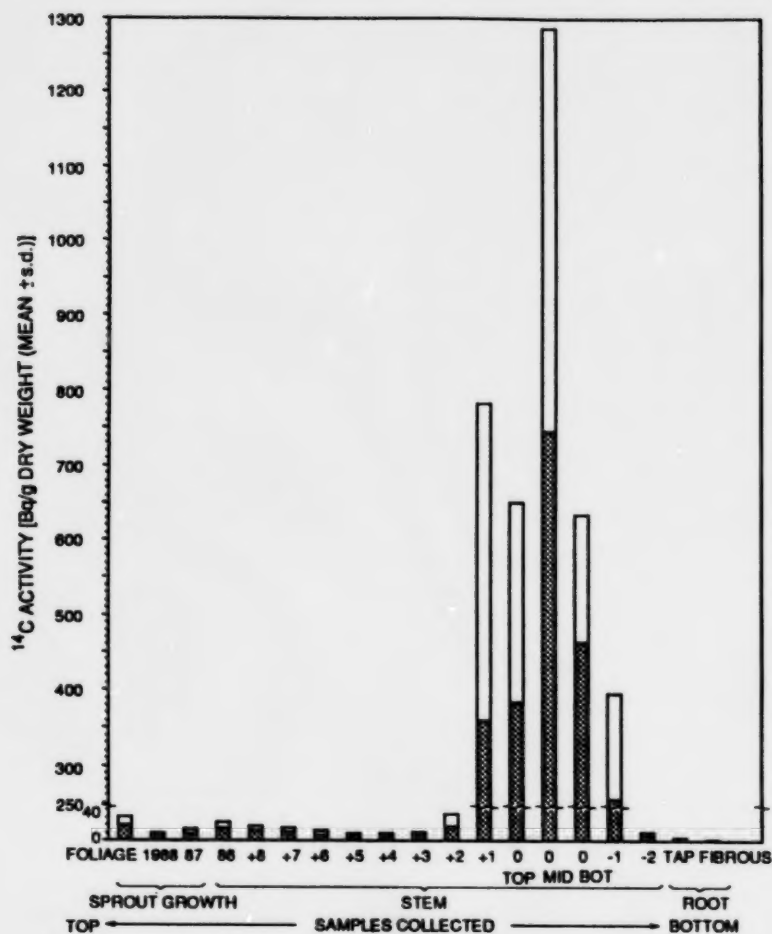


FIGURE 6

DISTRIBUTION OF ^{14}C ACTIVITY (EXPRESSED AS Bq/g DRY WEIGHT) IN STEM, ROOT AND FOLIAGE SAMPLES HARVESTED 500 DAYS AFTER TRUNK INJECTION OF SILVER MAPLE SAPLINGS WITH ^{14}C -UNICONAZOLE

Shaded bar represents mean value, and open bar mean + s.d. ($n = 3$). Detection limit was 0.4 Bq/g dry weight. Note break on vertical axis and change of scale

TABLE 9

Concentration of Uniconazole Calculated from ^{14}C Activity Data, in Foliage and Sprout Growth Samples Collected from Silver Maple Saplings 0, 17, 134 and 500 Days After Trunk Injection with ^{14}C -Uniconazole (Harvest Experiment)*

Sample	Days after Injection			
	0	17	134	500
	----- μM ($\mu\text{g/g}$ dry weight) -----			
Foliage	0.0 (0.0)	4.7 (2.9)	10.9 (6.8)	4.6 (2.9)
Sprout Growth 1988	--	--	--	3.8 (0.8)
Sprout Growth 1987	--	--	4.8 (1.0)	6.2 (1.3)
Sprout Growth 1986	0.6 (0.1)	25.2 (5.1)	6.7 (1.4)	11.6 (2.4)

* Uniconazole concentrations were estimated from ^{14}C Activity Data (Bq/g dry weight) as described in Materials and Methods. Mean values for ^{14}C activity in samples collected in the harvest experiment were used (n=3).

- No samples available for collection (day 0 was May 25th, 1987).

(C32)

other stem, root and foliage samples (see Figure 6). Note the break in the vertical axis and the change of scale. Data for days 17 and 134 were similar. At day 0 ^{14}C activity in the foliage was below the detection limit (data not shown). These data can be used to calculate the concentration of uniconazole in the various samples. The concentrations of uniconazole calculated for foliage and sprouts collected from saplings in the harvest experiment are shown in Table 9. Several assumptions were made in these calculations. The ^{14}C activity detected in a sample was assumed to indicate the presence of ^{14}C -uniconazole (and hence unlabelled uniconazole also, in the same ratio as in the solution injected into the sapling) rather than metabolites formed from uniconazole. Metabolites of uniconazole were found in foliage collected in the fall in both the foliage uptake and harvest experiments (see below) but it is not known if such metabolites have growth regulator properties. The presence of ^{14}C in metabolites would mean that the estimates of uniconazole concentration would be high. Distribution of ^{14}C activity (and thus uniconazole) in the various samples was assumed to be uniform, however Sterrett (1988) reported more ^{14}C activity in the xylem than in the phloem of apple stems injected with ^{14}C -uniconazole, and thus the concentrations shown in Table 9 are probably underestimates and do not reflect local levels. The concentrations are expressed either as μM uniconazole or μg uniconazole/g dry weight. Fresh foliage samples contain more water (relative to dry weight) than sprout (wood) samples and thus the relationship between the two expressions of concentration differ for the two sorts of sample. The concentrations of uniconazole calculated for foliage from saplings in the harvest experiment ranged from 4.6 to 10.9 μM (2.9 to 6.8 $\mu\text{g/g}$ dry weight) and in sprouts they ranged from 3.8 to 25.2 μM (0.8 to 5.1 $\mu\text{g/g}$ dry weight) see Table 9). The results for 1986 sprouts and foliage at day 0 are very low, and questionable given the fact that the samples were obtained immediately after the saplings were injected and there would have been no time for translocation to have occurred. Around the injection site the concentrations of uniconazole (expressed either way) were extremely high (data not shown). Sterrett (1988) reported that 5 μg uniconazole/g dry weight was found in apple shoot tissue (foliage plus sprouts) that exhibited growth inhibition. With the exception of foliage from 134 days and 1986 sprouts at 17 days (6.8 and 5.1 $\mu\text{g/g}$ dry weight respectively) the calculated values presented in Table 9 are less than this reported value. If the concentration of uniconazole has to be around the 5 $\mu\text{g/g}$ dry weight level before growth inhibition results, then this might explain the lack of obvious inhibition of sprout growth observed in the present study. Izumi et al (1985) reported that uniconazole at a concentration of 0.3 μM inhibited gibberellin biosynthesis in cell-free extracts of Curcubita maxima liquid endosperm. The concentrations presented in Table 9 are ten-fold or greater than this and thus the lack of obvious inhibition appears surprising. However, the concentrations given in Table 9 are somewhat inaccurate given the methodology used and the assumptions made. Gibberellin biosynthesis in cell-free extracts of Curcubita maxima may be more sensitive to uniconazole than sprout growth in silver maple saplings.

3.4 Metabolism of ^{14}C -Uniconazole in Foliage Collected from Silver Maple Saplings in the Fall of 1987 and 1988

In the results from the present study discussed above, ^{14}C activity in foliage, stem and root samples was described. In order to determine what proportion of the ^{14}C activity detected was present in authentic

TABLE 10

Distribution of ^{14}C Activity in Extracts of
Foliage from Silver Maple Saplings Trunk
Injected with ^{14}C - Uniconazole (Foliage Uptake Experiment)*

Fraction on Chromatography Plate Counted (Rf Values)	<u>Days After Injection</u>			
	----- 133 -----		----- 497 -----	
	Extract	Uniconazole Standard	Extract	Uniconazole Standard
	^{14}C Activity	^{14}C Activity	^{14}C Activity	^{14}C Activity
	----- % ^{14}C Activity on Plate -----			
0 - 0.1	11.0	0.1	5.5	0.1
0.1 - 0.2	13.2	0.0	8.7	0.0
0.2 - 0.3	5.9	0.1	2.9	0.0
0.3 - 0.4	3.3	0.1	0.7	0.0
0.4 - 0.5	3.5	0.1	3.0	0.0
0.5 - 0.6	3.6	0.1	3.8	0.0
0.6 - 0.7	3.1	0.1	3.3	0.1
0.7 - 0.8	20.9	10.7	17.7	8.5
0.8 - 0.9	32.2	87.8	53.1	90.1
0.9 - 1.0	3.5	1.1	1.9	1.2

* Extracts were prepared from foliage collected from the four saplings in the foliage uptake experiment (sapling J4 excluded) - values given are the means of the four extracts

(C32)

TABLE 11

Distribution of ^{14}C Activity in Extracts of
Foliage from Silver Maple Saplings Trunk
Injected with ^{14}C - Uniconazole (Harvest Experiment)*

Fraction on Chromatography Plate Counted (Rf Values)	<u>Days After Injection</u>			
	----- 134 -----		----- 500 -----	
	Extract	Uniconazole Standard	Extract	Uniconazole Standard
	^{14}C Activity	^{14}C Activity	^{14}C Activity	^{14}C Activity
	----- ^{14}C Activity on Plate -----			
0 - 0.1	10.7	0.1	11.4	0.2
0.1 - 0.2	13.5	0.0	7.0	0.1
0.2 - 0.3	4.6	0.0	1.5	0.1
0.3 - 0.4	2.9	0.0	0.9	0.0
0.4 - 0.5	3.4	0.0	5.4	0.0
0.5 - 0.6	5.0	0.0	8.5	0.0
0.6 - 0.7	2.6	0.1	4.2	0.1
0.7 - 0.8	11.3	23.9	6.7	20.7
0.8 - 0.9	39.6	74.4	47.9	77.6
0.9 - 1.0	6.5	1.3	6.6	1.2

* Extracts were prepared from foliage collected from the three saplings in the harvest experiment - values given are the means of the three extracts

(C32)

^{14}C -uniconazole rather than in metabolites of uniconazole, extracts were prepared from foliage samples collected in the fall of 1987 and 1988 and their components separated by thin-layer chromatography. The results from the foliage uptake experiment are given in Table 10. At day 133 approximately 53% of the ^{14}C activity in the extracts chromatographed with uniconazole, and by day 497 approximately 71% chromatographed with uniconazole. (The percentage ^{14}C activity in fractions 0.7 to 0.8 and 0.8 to 0.9 were combined in both cases, since the uniconazole standard was distributed between these two fractions.) Similar results were obtained in the harvest experiment, where 51% and 55% of the ^{14}C activity chromatographed with uniconazole in extracts of foliage collected at 134 and 500 days, respectively (see Table 11). In the studies of Sterrett and coworkers (Sterrett, 1985, 1988; Sterrett and Tworkoski, 1987) over 85% of the ^{14}C activity found in young shoot tissue after 27 days chromatographed with paclobutrazol, 75% of the ^{14}C activity found after 35 days chromatographed with flurprimidol and 92% of the ^{14}C activity found after 28 days chromatographed with uniconazole. In a more recent experiment (Sterrett, 1989) conducted for about 4 months, approximately 50% of the ^{14}C activity found in young shoots of apple chromatographed with uniconazole. This would suggest a direct correlation between shoot (foliage) age and the amount of metabolites found. In contrast to the low rates of metabolism described above, Early and Martin (1988) reported high rates in peach seedlings. By 9 days after treatment with labelled paclobutrazol was initiated, between 82 to 92% of the ^{14}C activity found in the leaves was not associated with the labelled paclobutrazol. The results from the present study would suggest that the site of metabolism is the foliage rather than the stem, because the percentage of ^{14}C activity not associated with uniconazole did not appear to increase from one year to the next. Sterrett (1988) reported more metabolite formation from uniconazole in young shoot tissue than in xylem stem tissue, although the experiment lasted 28 days only. A low rate of metabolism in the stem might explain the longevity of response in terms of inhibition of growth seen with uniconazole (Anonymous, 1988). There have been no reports on the rate of metabolism of growth regulators in the foliage of evergreen or deciduous species.

4.0 CONCLUSIONS

1. Trunk injection of silver maple saplings with ^{14}C -uniconazole in May 1987 resulted in an increase in chlorophyll pigmentation in the leaves and the appearance of 'stacked' leaves in the 1988 growing season. Such responses were non-uniform within saplings.
2. Treatment with uniconazole did not result in a large-scale inhibition of sprout growth. Sprout growth was inhibited in 1988 but only when median sprout lengths were considered and not mean sprout lengths. The lack of a large inhibition of sprout growth was probably the result of insufficient uniconazole being applied.
3. Over the course of the 1987 growing season there was an increase in the ^{14}C activity detected in the silver maple foliage. In 1988 there was a similar increase, although the maximum concentration reached was only about 1/3 that reached the previous year. Approximately 7.1 and 2.3% of the total ^{14}C -activity injected into the saplings was present in senescing foliage harvested in the fall of 1987 and 1988, respectively.

4. As the leaves turned from green/yellow to yellow in the fall of 1988 there was no apparent decrease in their ^{14}C activity content, suggesting no withdrawal of growth regulator at the same time as chlorophyll was being broken down.
5. In foliage collected in the fall of 1987 approximately 47% of the ^{14}C activity detected was not present in uniconazole, but in metabolites formed from uniconazole. In 1988 the percentage of ^{14}C activity discovered in metabolites was similar, suggesting that metabolism of uniconazole occurred in the foliage rather than in the stem around the injection site.
6. Immediately following trunk injection and by 134 or 500 days later, most of the ^{14}C activity detected in the saplings was around the injection sites. As described above some ^{14}C activity was present in the foliage, but little in the stem components situated between the injection site and the leaves.

ACKNOWLEDGEMENTS

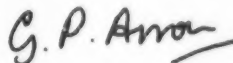
^{14}C -uniconazole was provided by Chevron Chemical Company. Dr. J.P. Sterrett (U.S.D.A./A.R.S. Frederick, MD) provided the miniature pressure injector used in this study. I would like to thank Dr. F.S. Spencer for helpful discussions and Ms. C. Thomson, Ms. J. Demeester, Ms. L. Vereecken-Sheehan, Mr. A. Spring, Mr. S. Poulton and, in particular, Ms. L. Bremner for expert technical assistance.

Approved by:



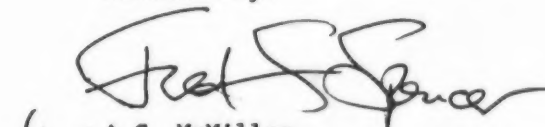
D.J.R. Dodd
Manager
Chemical Research Dept

Prepared by:



G.P. Arron
Associate Research Scientist
Biological Research Section

Reviewed by:


for A.C. McMillan
Supervising Biologist
Biological Research Section

GPA:ds

LITERATURE CITED

- Anonymous, 1986, Prunif Tree Growth Regulator. Technical Information Bulletin. Chevron Chemical Company.
- Aron, Y., Monselise, S.P., Goren, R., and Costo, J., 1985, Chemical control of vegetative growth in citrus trees by paclobutrazol. Hort Sci 10: 96-98.
- Arron, G.P., 1985 Effect of trunk injection of three growth regulators on sprout growth in silver maple. J. of Arbor 11: 301-306.
- Arron, G.P., 1986 Effect of Trunk Injection of Flurprimidol and Paclobutrazol on Sprout Growth in Silver Maple. J. of Arbor 12: 233-236.
- Camacho, F., and Arron, G.P., 1989 Effects of the Growth Regulators Paclobutrazol and Flurprimidol on the Growth of Terminal Sprouts formed on Trimmed Silver Maple Trees. Can. J. Stat (submitted).
- Dalziel, J. and Lawrence, D.K., 1984 Biochemical and Biological Effects of Kaurene Oxidase Inhibitors, such as Paclobutrazol. In: Menhenett R., D.K. Lawrence (eds) Biochemical Aspects of Synthetic and Naturally Occurring Plant Growth Retardants. British Plant Growth Regulator Group Monograph No 11, Wantage, U.K. p 43-57.
- Domir, S.C. and Roberts, B.R. 1983 Tree growth retardation by Injection of Chemicals. J. of Arbor 9: 217-224.
- Early, J.D. and Martin, G.C., 1988 Translocation and Breakdown of ¹⁴C-labeled Paclobutrazol in 'Nemaguard' Peach Seedlings. Hort Sci 23: 196-200.
- Gilliam, C.H., Fare, D.C. and Eason, J.T., 1988 Control of Acer Rubrum Growth with Flurprimidol. J of Arbor 14: 99-101.
- Greene, D.W., 1986 Effect of Paclobutrazol and Analogs on Growth, Yield, Fruit Quality, and Storage Potential of 'Delicious' Applies. J. Amer Soc Hort Sci 111: 328-332.
- Hare, R.C., 1984 EL-500: An Effective Growth Retardant for Dwarfing Southern Pine Seedlings. Can J For Res 14: 123-127.
- Hoadley, R.,B., 1980 Understanding Wood, Taunton Press, Newtown, Ct. 256 p.
- Houde, C., 1988 Basic Research with Tree Growth Regulators. Proceedings of the Third Annual Tree Growth Regulator Symposium. San Diego, March 1-3, 1988, p 9-14.
- Izumi, K., Kamiya, Y., Sakurai, A., Oshio, H. and Takahashi, N., 1985 Studies of Sites of Action of a New Plant Growth Retardant (E) 1 - (4 Chlorophenyl)-4,4-dimethyl-2- (1,2,4-triazol-1-yl)-1-penten-3-01) (S-3307) and Comparative Effects of its stereoisomers in a cell-free system from Cucurbita maxima Plant Cell Physiol 26: 831-827.
- Marini, R.P., 1968 Growth and Cropping of 'Redhaven' Peach Trees following

Foliar Applications of Flurprimidol and Paclobutrazol. J. Amer Soc Hort Sci 111: 849-853.

Miller, S.S., 1982 Growth and Branching of Apple Seedlings as influenced by Pressure-injected Plant Growth Regulators. Hort Sci 16: 775-776.

Peterson, J.I., 1969 A Carbon Dioxide Collection Accessory for the Rapid Combustion Apparatus for Preparation of Biological Samples for Liquid Scintillation Analysis. Anal Biochem 31: 189-201.

Richardson, P.J. and Quinlan, J.D., 1986 Uptake and Translocation of Paclobutrazol by Shoots of M.26 Apple Rootstock. Plant Growth Regulat 4: 347-356.

Spencer, F.S., 1989 Personal Communication to G.P. Arron.

Steffens, G.L., 1988 Gibberellin Biosynthesis Inhibitors: Comparing Growth-Retarding Effectiveness on Apple., J. Plant Growth Regul 7: 27-36.

Sterrett, J.P., 1979 Injection Methodology for Evaluating Plant Growth Retardants. Weed Sci 27: 688-690.

Sterrett, J.P., 1985 Paclobutrazol: A Promising Growth Inhibitor for Injection into Woody Plants. J. Amer Soc Hort Sci 110: 4-8.

Sterrett, J.P., 1988 XE-1019: Plant Response, translocation, and metabolism. J. Plant Growth Regul 7: 19-26.

Sterrett, J.P., 1989 Personal communication to G.P. Arron.

Sterrett, J.P. and Creager, R.A., 1977 A Miniature Pressure Injector for Deciduous Woody Seedlings and Branches. Hort Sci 12: 156-158.

Sterrett, J.P. and Tworkoski, T.J., 1987 Flurprimidol: Plant Response, Translocation and Metabolism. J. Amer Soc Hort Sci 112: 341-345.

Wang, S.Y., Sun, T. and Faust, M., 1986 Translocation of Paclobutrazol, a Gibberellin Biosynthesis Inhibitor in Apple Seedlings. Plant Physiol 82: 11-14.

Watson, M.R., 1987 Use of Tree Growth Regulators at Potomac Edison. J of Arbor 13: 65-69.

Watson, M.R., and Johnston, P.A., 1987 Tree Growth Regulators - One Company's Experience. Proceeds of the Fourth Symposium on Environmental Concerns in Rights-of-Way Management, Indianapolis, IN, October 25-28, 1987, p 540-549.

Wood, B.W., 1988 Paclobutrazol Suppresses Vegetative Growth of Large Pecan Trees. Hort Sci 23: 341-343.

APPENDIX A

Dry weight of 30 leaf samples collected in 1987 and 1988 from silver maple saplings trunk injected with ^{14}C -uniconazole

Day After Injection	Control (mean $\pm$ s.d.) (g)	Uniconazole (mean $\pm$ s.d.) (g)	Uniconazole* (mean $\pm$ s.d.) (g)
0	6.38 $\pm$ 0.56	8.03 $\pm$ 2.22	8.21 $\pm$ 2.53
7	4.73 $\pm$ 0.92	5.71 $\pm$ 0.77	5.80 $\pm$ 0.86
17	5.66 $\pm$ 1.29	6.69 $\pm$ 0.53	6.76 $\pm$ 0.59
25	8.29 $\pm$ 1.08	8.48 $\pm$ 0.92	8.56 $\pm$ 1.04
35	7.22 $\pm$ 0.80	8.13 $\pm$ 0.84	8.04 $\pm$ 0.94
45	7.51 $\pm$ 1.43	7.66 $\pm$ 0.76	7.82 $\pm$ 0.78
65	7.30 $\pm$ 1.68	7.59 $\pm$ 1.33	7.88 $\pm$ 1.33
85	6.12 $\pm$ 1.39	8.58 $\pm$ 1.36	8.94 $\pm$ 1.26
101	7.76 $\pm$ 1.51	8.59 $\pm$ 1.74	9.18 $\pm$ 1.34
122	7.10 $\pm$ 1.80	8.57 $\pm$ 1.41	9.19 $\pm$ 0.34
133	7.69 $\pm$ 2.05	8.27 $\pm$ 1.70	8.82 $\pm$ 1.35
375	2.81 $\pm$ 0.61	3.31 $\pm$ 0.45	3.20 $\pm$ 0.44
416	4.08 $\pm$ 0.56	5.59 $\pm$ 1.12	5.60 $\pm$ 1.30
444	4.70 $\pm$ 0.81	4.69 $\pm$ 0.61	4.91 $\pm$ 0.43
472	5.43 $\pm$ 1.43	5.40 $\pm$ 0.40	5.31 $\pm$ 0.39
497	6.17 $\pm$ 1.44	7.16 $\pm$ 1.91	7.38 $\pm$ 2.13

* Sapling J4 excluded from mean calculation (n=4).

(C32)

APPENDIX B

Proportion of ^{14}C activity found in the foliage of silver maple saplings trunk injected with ^{14}C -Uniconazole (Foliage Uptake Experiment) - Sapling Values*

Treatment	Sapling	1987	1988	1987	1988	Total dry weight of leaves collected (g)	Total ^{14}C activity (Bq)	1987	1988	1987	1988	^{14}C activity in leaves collected as function of amount injected (%)
Control	H2	867	1273	177	186	177	-25	7	-	-	-	-
"	J7	624	861	109	102	109	-13	-4	-	-	-	-
"	I5	1104	1846	237	239	237	-40	-150	-	-	-	-
"	J1	747	854	88	81	88	0	1	-	-	-	-
"	K1	767	870	140	116	140	-6	-80	-	-	-	-
Uniconazole	I6	812	1194	208	200	208	9279	2213	7.3	7.3	1.7	1.7
"	I3	1042	1340	193	191	193	6411	2750	5.0	5.0	2.2	2.2
"	J3	690	606	160	106	160	10917	2959	8.5	8.5	2.3	2.3
"	K4	654	780	129	127	129	10165	3967	7.9	7.9	3.1	3.1
"	J4	604	612	105	101	105	188	9	0.1	0.1	0.0	0.0

*Foliage samples were collected at various times in the 1987 and 1988 growing seasons (See Materials and Methods), including a complete strip of the foliage from the saplings at each fall, and the total amount of ^{14}C activity in that foliage expressed as a percentage of the ^{14}C activity injected into the saplings on May 25th, 1987.

(C32)

APPENDIX C

Proportion of ^{14}C activity found in the foliage of silver maple saplings trunk injected with ^{14}C -uniconazole (harvest experiment) - Sapling values*.

Treatment	Sapling	Days after injection	Total number leaves collected	Total dry weight (g)	Total ^{14}C activity (Bq)	^{14}C activity in foliage as function of amount injected (%)
Control	C2	0	493	64	5	-
"	E4	0	454	48	0	-
"	G8	0	327	52	-7	-
"	C3	134	565	247	-34	-
"	F6	134	944	265	25	-
"	E8	134	1139	304	25	-
Uniconazole	F4	0	519	50	0	0.0
"	B7	0	382	61	6	0.0
"	E7	0	527	108	-5	0.0
"	A3	17	465	71	2724	2.1
"	C5	17	551	109	875	0.7
"	D3	17	661	130	2023	1.6
"	C4	134	661	158	9480	7.4
"	C6	134	968	225	10980	8.6
"	F5	134	999	187	6767	5.3
"	B3	500	1484	275	2123	1.7
"	B5	500	1284	119	3089	2.4
"	F1	500	1182	132	3584	2.8

* Foliage was collected from the saplings as they were harvested on the days indicated.

(C32)